

SEQUENCE LISTINGS

<110> Hanmi Pharm. Co., Ltd.

<120> EXPRESSION VECTOR FOR SECRETING ANTIBODY FRAGMENT USING E. COLI SIGNAL SEQUENCE AND METHOD FOR MASS-PRODUCING ANTIBODY FRAGMENT

<130> Q94300

<140> 10/576,068

<141> 2006-04-14

<150> KR1020030072216

<151> 2003-10-16

<150> PCT/KR04/02625

<151> 2004-10-14

<160> 36

<170> KopatentIn 1.71

<210> 1

<211> 75

<212> DNA

<213> Artificial Sequence

<220>

<223> gene fragment of light chain variable region

<400> 1

gggaagcttc gatcggacat ccagatgacc cagtctccat cctccctgtc tgcattctgta 60

ggggacagag tcacc 75

<210> 2

<211> 80

<212> DNA

<213> Artificial Sequence

<220>

<223> gene fragment of light chain variable region

<400> 2

tggtttttgc tgataccagg ctaagtaatt tctgatgccc tgacttgccc gacaagtgat 60

ggtgactctg tcccctacag 80

<210> 3

<211> 80

<212> DNA

<213> Artificial Sequence

<220>

<223> gene fragment of light chain variable region

<400> 3
cctggtatca gcaaaaacca gggaaagccc ctaagctcct gatctatgct gcatccactt 60
tgcaatcagg ggtcccatct 80

<210> 4
<211> 80
<212> DNA
<213> Artificial Sequence

<220>
<223> gene fragment of light chain variable region

<400> 4
aggctgtagg ctgctgatgg tgagagtga atctgtccca gatccactgc cactgaaccg 60
agatgggacc cctgattgca 80

<210> 5
<211> 80
<212> DNA
<213> Artificial Sequence

<220>
<223> gene fragment of light chain variable region

<400> 5
ccatcagcag cctacagcct gaagatgttg caacttatta ctgtcaaagg tataaccgtg 60
caccgataac ttttggccag 80

<210> 6
<211> 41
<212> DNA
<213> Artificial Sequence

<220>
<223> gene fragment of light chain variable region

<400> 6
tttgatttcc accttggtcc cctggccaaa agtatacggt g 41

<210> 7
<211> 75
<212> DNA
<213> Artificial Sequence

<220>
<223> gene fragment of heavy chain variable region

<400> 7
 gggaagcttc gatcggaggt gcagctgggtg gagtctgggg gaggcttggt acagcccggc 60
 aggtccctga gactc 75

<210> 8
 <211> 79
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> gene fragment of heavy chain variable region

<400> 8
 agcttgccgg acccagtgc tggcataatc atcaaagggtg aatccagagg ccgcacagga 60
 gagtctcagg gacctgccg 79

<210> 9
 <211> 80
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> gene fragment of heavy chain variable region

<400> 9
 tgcactgggt cgggcaagct ccagggaagg gcctggaatg ggtctcagct atcacttgga 60
 atagtgggtca catagactat 80

<210> 10
 <211> 80
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> gene fragment of heavy chain variable region

<400> 10
 atacagggag ttcttggcgt tgtctctgga gatgggtgaat cggccctcca cagagtccgc 60
 atagtctatg tgaccactat 80

<210> 11
 <211> 80
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> gene fragment of heavy chain variable region

<400> 11
 acgccaaagaa ctccctgtat ctgcaaata acagtctgag agctgaggat acggccgtat 60
 attactgtgc gaaagtctcg 80

<210> 12
 <211> 84
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> gene fragment of heavy chain variable region

<400> 12
 cactcgagac ggtgaccagg gtaccttggc cccaatagtc aagggaggac gcggtgctaa 60
 ggtacgagac ttctgcacag taat 84

<210> 13
 <211> 39
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> RT-PCR forward primer specific for heavy chain

<400> 13
 cccaagctta ggcctccacc aagggcccat cggctcttc 39

<210> 14
 <211> 48
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> RT-PCR reverse primer specific for heavy chain

<400> 14
 gggggatcct tatgggcacg gtgggcatgt gtgagttttg tcacaaga 48

<210> 15
 <211> 42
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> RT-PCR forward primer specific for light chain

<400> 15

cccaagcttt cgcgaactgt ggctgcacca tctgtcttca tc 42

<210> 16
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> RT-PCR reverse primer specific for light chain

<400> 16
cccggatccc taacactctc ccctgttgaa gctctttgtg ac 42

<210> 17
<211> 69
<212> DNA
<213> modified E. coli thermostable enterotoxin II signal sequence

<400> 17
atgaaaaaga caatcgcat tcttcttgca tctatgttcg ttttttctat tgctacaaat 60
gcccaggcg 69

<210> 18
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> forward primer containing StuI restriction enzyme site

<400> 18
tctattgcta caaatgccca ggccttccca accattccct tatcc 45

<210> 19
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> reverse primer containing StuI restriction enzyme site

<400> 19
agataacgat gtttacgggt ccggaagggt tggttaaggga atagg 45

<210> 20
<211> 51
<212> DNA
<213> Artificial Sequence

<220>

<223> reverse primer specific for light chain

<400> 20
gggggatcct cacgcggcgc atgtgtgagt tttgtcacia gatttaggct c 51

<210> 21
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> forward primer containing SD sequence and BamHI restriction enzyme site

<400> 21
gggggatcca ggaggtgatt tatgaaaaag acaatcgcat ttc 43

<210> 22
<211> 44
<212> DNA
<213> Artificial Sequence

<220>
<223> forward primer containing BpuI restriction enzyme site

<400> 22
ggggctgagc aggaggtgat ttatgaaaaa gacaatcgca tttc 44

<210> 23
<211> 52
<212> DNA
<213> Artificial Sequence

<220>
<223> reverse primer containing BpuI restriction enzyme site

<400> 23
ggggctcagc tcacgcggcg catgtgtgag ttttgtcaca agatttaggc tc 52

<210> 24
<211> 63
<212> DNA
<213> E. coli OmpA signal sequence

<400> 24
atgaaaaaga cagctatcgc gattgcagtg gcactggctg gtttcgctac cgttgcgcaa 60

gct 63

<210> 25
<211> 30

<212> DNA
 <213> Artificial Sequence

 <220>
 <223> forward primer specific for heavy chain

 <400> 25
 gaggttcagc tagtcgagtc aggaggcggc 30

 <210> 26
 <211> 51
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> forward primer containing HindIII and StuI restriction enzyme sites

 <400> 26
 gggagatctt cacgcggcgc atgtgtgagt tttgtcaca gatttaggct c 51

 <210> 27
 <211> 30
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> reverse primer containing stop codon and BamHI restriction enzyme site

 <400> 27
 gacattcaaa tgaccagag cccatccagc 30

 <210> 28
 <211> 42
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> forward primer containing HindIII and NruI restriction enzyme sites

 <400> 28
 cccagatctc taacactctc ccctgttgaa gctctttgtg ac 42

 <210> 29
 <211> 41
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> reverse primer containing stop codon and BamHI restriction enzyme site

<400> 29
 ggggtcgaca ggaggtgatt tatgaaaaag acagctatcg c 41

<210> 30
 <211> 51
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> reverse primer containing SalI restriction enzyme site

<400> 30
 ggggtcgact cacgcggcgc atgtgtgagt tttgtcacia gatttaggct c 51

<210> 31
 <211> 42
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> forward primer specific for modified E. coli enterotoxin II signal peptide and containing NdeI restriction enzyme site

<400> 31
 gggcatatga aaaagacaat cgcatttctt cttgcatcta tg 42

<210> 32
 <211> 705
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> TNF-alpha heavy chain

<400> 32
 gaggttcagc tagtcgagtc aggaggcggg ttggtacagc ccggcaggtc cctgagactc 60
 tcctgtgcgg cctctggatt cacctttgat gattatgcc a tgactgggt ccggcaagct 120
 ccagggaagg gcctggaatg ggtctcagct atcacttgga atagtggta catagactat 180
 gcggactctg tggagggccg attcaccatc tccagagaca acgccaagaa ctccctgtat 240
 ctgcaaatga acagtctgag agctgaggat acggccgtat attactgtgc gaaagtctcg 300
 taccttagca ccgcgtctc ccttgactat tggggccaag gtaccctggg caccgtctcg 360
 agtgcccca ccaagggccc atcgggtctc ccctggcac cctcctccaa gagcacctct 420
 gggggcacag cgccctggg ctgcctgggc aaggactact tccccgaacc ggtgacggtg 480
 tcgtggaact caggcgcctt gaccagcggc gtgcacacct tcccggctgt cctacagtcc 540

tcaggactct actccctcag cagcgtggtg accgtgccct ccagcagctt gggcaccag	600
acctacatct gcaacgtgaa tcacaagccc agcaacacca aggtggacaa gaaagttgag	660
cccaaattctt gtgacaaaac tcacacatgc ccaccgtgcc catag	705

<210> 33
 <211> 645
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> TNF-alpha light chain

<400> 33	
gacatccaga tgaccagtc tccatcctcc ctgtctgcat ctgtagggga cagagtcacc	60
atcacttgtc gggcaagtca gggcatcaga aattacttag cctgggtatca gcaaaaacca	120
gggaaagccc ctaagctcct gatctatgct gcatccactt tgcaatcagg ggtcccatct	180
cggttcagtg gcagtggatc tgggacagat ttcactctca ccatcagcag cctacagcct	240
gaagatggtg caacttatta ctgtcaaagg tataaccgtg caccgtatac ttttggccag	300
gggaccaagg tggaatcaa acgaactgtg gctgcaccat ctgtcttcat cttcccgcc	360
tctgatgagc agttgaaatc tggaactgcc tctgttgtgt gcctgctgaa taacttctat	420
cccagagagg ccaaagtaca gtggaagggtg gataacgccc tccaatcggg taactcccag	480
gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgacg	540
ctgagcaaag cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc	600
ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt gttag	645

<210> 34
 <211> 7
 <212> PRT
 <213> TNF-alpha light chain

<400> 34
 Asp Ile Gln Met Thr Gln Ser
 1 5

<210> 35
 <211> 8
 <212> PRT
 <213> TNF-alpha heavy chain

<400> 35
 Glu Val Gln Leu Glu Val Asp Ser
 1 5

<210> 36
 <211> 12
 <212> PRT
 <213> N-terminal sequence of recombinant TNF-alpha

 <400> 36
 Asp Glu Ile Val Gln Met Leu Thr Val Gln Asp Ser
 1 5 10